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Role of Digestive Gland in the Energetic Metabolism of *Penaeus setiferus*

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Abstract. We determined the role of the digestive gland in the respiratory metabolism of *Penaeus setiferus* adult males as a step toward proposing a feeding schedule based on the cycle of activity in the digestive gland. We measured pre- and postprandial values for oxygen consumption rate and hemolymph glucose concentrations in live animals, and oxygen consumption rate and glycogen concentration in excised digestive gland. After the animals were fed, which enhanced general metabolic activity, these indices changed. There was a high correlation between the oxygen consumption rate of the animal and the glucose concentration in the hemolymph, and between the oxygen consumption rate by the digestive gland and the glycogen concentration in the digestive gland, all in relation to time after feeding. Correlations support the hypothesis that the energy demand depends upon the metabolic substrate concentration. In this theory, glucose sustains muscle activity (during ingestion of food) and glycogen is the product of the digestive gland during food assimilation. Our observations of metabolic dynamics during the feeding period allowed us to examine the feeding process. The metabolic activity of the digestive gland was highest 6 h after feeding. This could mean that assimilation, having started 2 h after food intake, peaked 6 h after feeding. Eight hours after feeding, the oxygen consumption rate of the digestive gland decreased and fell to values similar to those recorded for animals subjected to 72 h of fasting.

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Abbreviations AHI, apparent heat increment; afdw, ash-free dry weight; dw, dry weight.

Introduction

The digestive gland (also known as the midgut gland or hepatopancreas) of decapod crustaceans serves the dual role of secreting enzymes and absorbing digested food. This gland is composed of embryonic (E) cells, which give rise to two basic cell types: R cells (Restzellen), which store nutrients, and F cells (Fibrillenzellen), which secrete enzymes (Hirsch and Jacobs, 1930). The F cells develop into B cells (Blasenzellen), a more mature secretory stage with a large vacuole containing digestive enzymes (Gibson and Barker, 1979). The overall functions of the digestive gland, including the temporal relationship of secretion and absorption to food intake, have been assessed in several species. Because many of those studies used histochemical methods, the results are difficult to interpret.

Gibson and Barker (1979) reported that in the digestive gland of *Homarus americanus*, B cells were replaced 12 h after food ingestion, and in *Penaeus semisulcatus* the highest activity of proteolytic enzymes was evident within 7 to 10 h. Al-Mohanna and Nott (1987) detected in the latter species a cycle of maximum enzymatic activity 6 h after food intake, with production of feces containing B cells, membranous remains, and particulate matter after 24 h. Hopkin and Nott (1980) found that in *Carcinus maenas*, digestion and absorption took about 12 h after feeding and were followed by an exeretory phase lasting from about 12 to 48 h after feeding.

Despite the amount of information published on the activity and characteristics of the crustacean digestive gland, little is known about its role in respiratory activity during feeding. Several authors (*e.g.*, Beamish and Trippel, 1990) recognized that the apparent heat increment (AHI;

previously referred to as specific dynamic action, SDA) is an indicator of the mechanical and biochemical processes associated with the ingestion and assimilation of food. Although muscular tissue is responsible for the mechanical activity, the digestive gland is the site of metabolic functions that break the stomach contents down biochemically. Hence, the AHI may result from addition of the energy used in the above two processes; this constitutes a considerable percentage of the daily energy budget in aquatic organisms (Du-Preez *et al.*, 1992; Chakraborty *et al.*, 1993).

In aquaculture, AHI has been used in the selection of diets for raising shrimp; thus it is imperative to determine the magnitude of energy costs associated with feeding activity. No previous studies have correlated this energy cost to digestive gland metabolism during food ingestion. Therefore, no approximations have been made that allow the differentiation of components of the AHI and the role of the digestive gland in these processes. Our study was aimed at determining the role of the digestive gland in the respiratory metabolism of *Penaeus setiferus*. At various stages while the shrimp were ingesting and assimilating food, we measured the rate of oxygen consumption in live animals and in the digestive gland; the content of glucose in hemolymph; and the content of glycogen in the digestive gland.

Materials and Methods

Animals

Thirty-nine sexually mature male shrimp (*P. setiferus;* 37.57 ± 0.54 g wet weight) were caught on the continental shelf off Laguna de Términos, Campeche, México. In the laboratory, the shrimp were placed in 1000-l flow-through tanks, with aerated seawater, under a light/dark cycle of 14/10 h. After 24 h of conditioning, shrimp were left without food for 72 h to provide fasting conditions. During the experiment, salinity was kept at 32‰ and temperature at 28 ± 1°C.

Oxygen consumption rate in whole live shrimp

After the fasting period, 6 shrimp were placed in a 1-l chamber connected to a flow-through respirometer (0.1 l/min) (Martinez-Otero and Díaz-Iglesia, 1975), in which they were acclimated for 8 h before the experiments were conducted. Oxygen consumption rate was estimated by the difference in oxygen concentration in the input and output of the chamber. The difference was multiplied by the flow rate and corrected for a control chamber without organisms. Metabolic rate was recorded at time 0 (animals fasting 72 h) and at 1, 2, 4, 6, and 24 h after a meal of 1 g squid meat (*Loligo brevis*) was given and totally ingested. These times were selected on the basis of the finding that the major activity of the digestive gland in *P. semisulcatus* occurs between 1 and 6 h after feeding (Al-Mohanna and Nott, 1987).

At the end of the experimental phase, all animals were sacrified and fresh weight, dry weight (dw), and ash-free dry weight (afdw) determined. Results of oxygen consumption measurements were expressed in milligrams of oxygen per gram per hour afdw (Sanchez *et al.*, 1991). AHI was estimated as the difference between feeding and fasting rates of oxygen consumption (Du Preez *et al.*, 1992). This difference was transformed using the exocaloric coefficient of 3.53 cal/mg O_2 consumed (Elliot and Davison, 1975), and expressed in relation to a mean afdw of 11.4 g/(animal · 24 h).

Digestive gland oxygen consumption rate

A total of 15 shrimp were used for this experiment. Fasted (72 h) animals were placed in a 600-l tank with filtered seawater. The digestive glands of animals chosen at random were dissected and placed in physiological solution for crustaceans (Prosser, 1973). This solution was made with NaCl (26.42 g/l), KCl (1.12 g/l), $CaCl_2$ (2.78 g/l), $MgCl_2$ (0.32 g/l), $MgSO_4$ (0.49 g/l), H_3BO_3 (0.53 g/l), and NaOH (0.192 g/l) with a pH of 7.6. Each digestive gland was cut in two, and each half was considered a duplicate of the other. Rate of oxygen consumption was measured in fasting shrimp (72 h) and at 1, 2, 6, and 8 h after feeding. Each piece of digestive gland was placed in a microrespirometer chamber with 2 ml of previously aerated physiological solution. The oxygen concentration in the chambers was measured, under gentle agitation, with a Strathkelvin Model 781 oxygen meter equipped with a high-sensitivity membrane (12.5 μm) electrode. This system was connected to a thermostat that kept temperature at 28 ± 0.01°C during the experiment. Measurements lasted for 3 to 5 min, recording oxygen variations every 10 s. Due to the uniformity of readings, only the results obtained 30 s after sectioning the digestive gland were used.

Glycogen concentrations in digestive gland and glucose in hemolymph

Glycogen was measured in digestive gland sections from 18 shrimp at time 0 (after 72 h fasting), and at 1, 2, 4, 6, and 24 h after feeding. Glycogen was extracted with anthrone reagent. This reagent consisted of a solution of 0.05% anthrone, 1% thiourea, and 72% H_2SO_4 (Carroll *et al.*, 1956). The digestive gland was first homogenized in trichloroacetic acid (TCA; 5%) for 3 min. After centrifugation (3000 rpm) the supernatant was filtered (acid-free paper) and quantified. This procedure was performed

Table I

Oxygen consumption rate (VO_2), blood glucose concentration, and digestive gland glycogen concentration of Penaeus setiferus *in relation to time after feeding*

	Intact animals		Digestive gland		
Time H	VO_2 mg O_2/(g afdw · h)	Glucose mmol/l	Wet weight g	VO_2 mg O_2/(g afdw · h)	Glycogen mg/100 g dw
0	1.01	1.66	0.62	1300	1.70
	(0.22)	(0.01)	(0.01)	(130)	(0.14)
1	1.56	5.46	1.28	1310	2.08
	(0.09)	(0.40)	(0.17)	(107)	(0.43)
2	1.25	5.71	1.02	1507	4.10
	(0.75)	(0.01)	(0.07)	(204)	(0.43)
4	1.45	5.67	0.80	—	12.06
	(0.19)	(0.19)	(0.01)		(0.84)
6	1.12	5.45	0.92	2027	17.41
	(0.10)	(0.02)	(0.05)	(112)	(0.14)
8	—	—	—	1250	—
				112	
24	0.93	1.28	0.59	—	0.74
	(0.07)	(0.01)	(0.06)		(0.06)
N by measurement	6	3	3	3	3
Total	6	18		15	

Values as mean. SEM in parentheses.

three times. One ml of TCA filtrate was pipetted into a Pyrex centrifuge tube and mixed with 5 volumes of 95% ethanol. The tubes were placed in a water bath at 37°C for 3 h. After precipitation occurred, the tubes were centrifuged at 3000 rpm for 15 min. The packed glycogen was dissolved by addition of 2 ml of distilled water. Ten ml of anthrone reagent was delivered into each tube with vigorous blowing, and the tubes were placed in a cold (4°C) tap water bath. Later all tubes were placed in a boiling water bath for 15 min. The contents of the tubes were transferred to a colorimeter tube and read at 620 nm after the instrument was adjusted with the reagent blank (distilled water plus anthrone reagent). A standard was prepared by adding 2 ml of standard glucose solution containing 0.1 mg of glucose to anthrone reagent.

Glucose concentration in the hemolymph

Glucose was measured in hemolymph from the same shrimp used for the glycogen determination. Before the digestive gland was excised, 200 μl of hemolymph was extracted from the pericardium of each shrimp. A 12.5% solution of sodium citrate was used to prevent clotting (Martin *et al.*, 1991). The glucose concentration in the hemolymph was measured with a commercial kit for medical diagnosis (Merckotest 3306, Rosas *et al.*, 1992a).

Statistical analysis

Analysis of variance (ANOVA) was used to test the significance of the results obtained. Duncan's multiple range test (Zar, 1974) was used to determine differences in the means of oxygen consumption of whole animals, oxygen consumption of digestive gland, glycogen concentration in digestive gland, and glucose concentration in hemolymph. For all groups, an analysis of covariance was performed between the rate of oxygen consumption by the animal and the concentration of glucose in hemolymph and between the rate of oxygen consumption by the digestive gland and the concentration of glycogen in the hemolymph.

Results

Respiratory metabolism and levels of glucose and glycogen changed with time after feeding (Table I). The oxygen consumption rate of live organisms was higher between 1 and 4 h after feeding ($p < 0.05$) than at time zero. A respiratory rate increase of 54% and an AHI of 1.95 cal/(g afdw · h), equivalent to 533.3 cal/(11.4 g afdw · day), were obtained (Table II). Daily AHI was 8.5% of the energy of the ingested food (Table II). Subsequently there was a reduction of about 28% in oxygen consumption rate (as observed at 6 h after feeding), and the oxygen consumption rate returned to the initial level by 24 h after feeding (Table I).

Digestive gland weight increased after 1 h, from 0.62 to 1.28 g dw/animal, then diminishing gradually in the 2 and 6 h observations. The lowest value was obtained 24 h after feeding (Table I). Digestive gland oxygen consump-

Table II

Apparent heat increment (AHI) calculated for Penaeus setiferus

	mg O_2/ (g afdw · h)	cal/ (g afdw · h)	cal/ (11.4 g afdw · h)
AHI	0.55 ± 0.03	1.95 ± 0.09	533.5 ± 26.7
AHI % of the energy of the ingested food	—	—	8.5

Values as mean ± SEM. Shrimp wet weight: 37.57 ± 0.51 g; shrimp ash-free dry weight: 11.4 ± 0.16 g; energy content of *Loligo brevis*: 6300 cal/g afdw.

tion rate remained constant between time zero and 1 h, with an average of 1305 mg O_2/(g dw · h) (Table I). A gradual increase was detected until it reached its highest level, 6 h after feeding, which was 56% higher than for fasting animals (Table I) ($p < 0.05$). The oxygen consumption rate of the digestive gland was returned to fasting levels 8 h after feeding.

Hemolymph glucose concentration showed a significant increase by 1 h after feeding (Table I). Recorded values were 1.66 mmol/l in starved animals and 5.46 mmol/l in fed shrimp. The hemolymph glucose level of fed shrimp remained stable between 1 and 6 h, the average value being 5.5 mmol/l. Twenty-four hours after feeding, glucose concentration had fallen to 1.28 mmol/l, observed in starved animals ($p < 0.05$).

Glycogen in digestive gland showed a gradual increase after 2 h of feeding, reaching a maximum 10.2 times larger than fasting animals at 6 h (Table I). Twenty-four hours after feeding, glycogen levels were significantly lower than those observed before feeding.

The oxygen consumption rate of the animal was correlated with hemolymph glucose ($r = 0.78$), and the oxygen consumption rate of the digestive gland was correlated with glycogen concentration ($r = 0.99$; Table III). In both cases, values of r and p confirm a positive relationship between responses, which are positive and linear ($p < 0.05$).

Discussion

The use of mature male shrimp in this study excludes the effect of biochemical processes related to gonadal maturation, thus assuring that the results were due solely to the activity of the digestive gland. In previous studies, Rosas *et al.* (1992a, b) showed that in a 24-h cycle, the oxygen consumption rate and the hemolymph glucose concentration of *P. setiferus* were highest between 9 and 16 h after feeding, which assures an 8-h interval of general metabolic stability. In the present study we used previous results to select a time period for observation of metabolic changes due to feeding, thus eliminating possible effects of circadian rhythm upon metabolic activity.

Apparent heat increment (AHI) is related to an increase in oxygen consumption rate induced by locomotory activity, capture, ingestion and digestion of food, and biochemical activity related to absorption of material (Beamish and Trippel, 1990). These expenditures of energy can constitute a high percentage of the energy used by shrimp. If we consider organisms with an average weight of 40 g dw (11.4 g afdw), a squid diet with a caloric value of 1890 cal/g afdw (Del Barco, 1975), and an AHI of 533.5 cal/(11.4 g afdw · day), it is possible to infer that the AHI corresponds to 8.5% of the daily metabolized energy (Table II). Although the AHI levels might change depending on the quality and quantity of food, our results can be applied to squid (*Loligo brevis*) diets normally given to reproductive shrimp. Du Preez *et al.* (1992) reported an AHI of 2.4% to 19.5% of ingested energy for juveniles of *Penaeus monodon* fed shrimp muscle, and 2% to 17% for shrimp fed with commercial balanced feed. In another study, Nelson *et al.* (1977) reported that in juvenile *Macrobrachium rosenbergii*, the AHI fluctuates from 7.4% to 27.5% of available energy, depending on the type of feed, with the highest level found in those fed on tubifid worms.

From the results of this study it is possible to isolate some components of the energy costs associated with AHI, and shed some light on utilization and assimilation (Table IV). Because of the difficulty in estimating each AHI component directly, we attempted to differentiate them on the basis of their respective times. Once food was provided, the animals displayed intensive muscular activity (pleopod motion), which contrasted with the no-motion behavior observed within the respirometer chamber during the 8-h acclimatization period. As the first three pairs of pereiopods secure the food, it is fragmented and passed onto the mouth parts for ingestion. Contact digestion then begins (Gibson and Barker, 1979; Al-Mohanna and Nott, 1987) (Table IV). This behavior occurred during the first hour after feeding and coincided with the elevation of hemolymph glucose concentration and oxygen consump-

Table III

Oxygen consumption rate (mg O_2/(g afdw · h)) and concentrations of hemolymph glucose (mmol/l) correlation (A) and digestive gland oxygen consumption rate (mg O_2/(g dw · h)) and digestive gland glycogen (mg/g) correlation (B) of Penaeus setiferus

	a	*b*	*r*	*p*<
A	0.83	0.09	0.78	0.05
B	1185.30	0.70	0.99	0.002

$Y = a + bX$. Values from all groups.

Table IV

Feeding schedule of Penaeus setiferus

Stage	Activity	Source	Associated time	Metabolic substrate
I	Excitation, Ingestion, and Contact digestion (Stomach)	Maximum VO_2 (AHI)	1	Glucose (5.5 mmol/l)
II	Absorption of small particles and Chyme digestion (Lumen)	Weight increment of DG VO_2 DG VO_2 AHI	1–2	Glucose (5.5 mmol/l) Proteins (?) Lipids (?)
III	Assimilation and Synthesis	Maximum VO_2 DG Glycogen (17.41 mg/100 g dw)	6	Glucose (5.5 mmol/l) Proteins (?) Lipids (?)
IV	Feces production and Digestive gland metabolic rate reduction		8	
V	General metabolic reduction		24	Less glucose than T_0 57% less glycogen than in T_0

VO_2 (AHI) is the oxygen consumption rate of whole animals; VO_2 DG is the digestive gland oxygen consumption rate; DG is the digestive gland. This schedule integrated all results obtained.

tion noted 1 h after feeding (Table I). Taking into account that the oxygen consumption of the digestive gland remained constant, we attribute the increase in oxygen consumption to the mechanical aspects of feeding (muscle excitement, ingestion, and contact digestion). During this time glycogen reserves in muscular tissue and digestive gland provide glucose in hemolymph as fuel for these activities. The correlation between oxygen consumption rate and glucose level in hemolymph reported for crustaceans in this and other works can be used as an indicator of this process (Table III) (Ramos and Fernandez, 1981; Brito and Diaz-Iglesia, 1987; Diaz-Iglesia *et al.*, 1987; Rosas *et al.*, 1992a).

Digestive gland weight increased as a function of time after feeding. A maximum weight of 1.28 g was reached 1 h after feeding; this value was twice as high as that recorded for fasting animals. If we attribute this difference in weight to the amount of food in the digestive gland (Al-Mohanna and Nott, 1987), we can evaluate the efficiency of incorporation of ingested squid. Considering that 1 g of food was available per shrimp and using initial weight of the digestive gland, we estimate an efficiency of 66% of ingested food. In view of this result and those reported by Al-Mohanna and Nott (1987), for aquaculture purposes it is the activity of the digestive gland rather than the ingestion of the food that should be considered in establishing a feeding schedule for *P. setiferus*.

Once the food is digested in the gut, the chyme and fine particles are digested in the lumen and absorbed by diffusion to the inner portions of the digestive gland tubules, thus initiating the accumulation of glycogen (Al-Mohanna and Nott, 1987; Hopkin and Nott, 1980). The 140% increase in the glycogen concentration in the digestive glands that took place 2 h after feeding could indicate the onset of glucogen synthesis (Tables I and IV). Because these processes require energy, we would expect the oxygen consumption of the digestive gland to increase. In fact, a 56% increase in oxygen consumption was recorded in the digestive gland of *P. setiferus* after 6 h (Table I). This increase can be correlated to the calorigenic effect induced by the food in the digestive gland. In this study, the oxygen consumption rate of the digestive gland was 1287% higher than that of intact animals. Although we have no explanation for such a high consumption rate, these results are similar to those obtained by other authors. Conceiçao (1993) and Diaz-Iglesia *et al.* (1995) recently found that in feeding *Panulirus argus*, the oxygen consumption rate of the digestive gland was 312% higher than that observed in living lobsters. The lack of endogenous controls during *in vitro* experiments could account for the high metabolic rate found for *Penaeus setiferus* and *Panulirus argus*. Schmidt-Nielsen (1984) stated that "the metabolic rate in homologous tissues (liver, for example) is relatively constant, irrespective of body size, but this rate is restricted or depressed in the large animals by some 'central' control or other 'organismic' factor resident in the intact organism." Although this observation was based on data for mammals, it might apply equally well to shrimp and explain metabolism-depressing factors in the digestive gland. Hormones from the eyestalks could also be responsible for the metabolic control of the digestive gland in living animals (Silverthorn, 1975a, b; Kleinholz, 1976; Madyastha and Rangneker, 1976; Mauviot and Castell, 1976; Radakrishnan and Vijakumaran, 1984; Rosas *et al.*, 1991). The presence of elevated glycogen levels concomitant with an increase in the oxygen consumption rate by the digestive gland may point to the synthesis of reserves during this period mirroring the as-

similation of ingested food (Table IV). This hypothesis is supported by the correlation between metabolic activity and glycogen concentrations (Table III).

Major activity of the digestive gland has been reported 6 h after feeding activity in *P. semisulcatus* (Al-Mohanna and Nott, 1987). This elapsed time could mirror the highest respiratory activity in the digestive gland of *P. setiferus* (Table I) and indicate that assimilation, having started 2 h after food intake, would peak 6 h after feeding. Eight hours later, the oxygen consumption of the digestive gland could decrease and fall to values similar to those recorded for digestive gland tissue from animals subjected to 72 h of fasting (Table I). Although the amount of energy lost as heat cannot be precisely accounted for in all the processes in this study, the largest amount of energy consumed was associated with the mechanical processes of feeding, as evidenced by the oxygen consumption of living animals 1–2 h after feeding (Table I).

The accumulation of glycogen as storage material can also be used as an indicator of the energetic potential of the diet, because glycogen is the source of glucose for metabolic use and for the synthesis of chitin (Gwing and Stevenson, 1979; Chan *et al.*, 1988). Considering that molting is an important factor in shrimp growth, the dynamics of glucose could be useful in determining the diet for shrimp species.

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